NOTES ON A SERIES OF INVESTIGATIONS INTO THE METHODS OF IMMUNISATION AGAINST DIPHTHERIA.

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An investigation into some aspect of the prevention of any disease requires no defence; when disease is so universal as diphtheria, the field in question holds many problems. It is not claimed that in this thesis new and original solutions to these problems are given; an attempt has been made to outline the possibilities and difficulties of one method of prophylaxis, and judge of its usefulness when compared with others. In doing this various facts have been noted -- some practical and others more academic -- and their significance is discussed.

Certain abbreviations have been used in this thesis. They are as follows:-

'S+'	=	Schick positive.
'S-'	=	" negative.
'M+'	=	Moloney positive.
'M-'	=	" negative.
'D+'	=	Dick positive.
'D-'	=	" negative.
'PS'	=	pseudo reaction.
T.A.M.	=	Toxoid Antitoxin Mixture.
T.A.F.	=	Toxoid Antitoxin Floccules.
F.T.	=	Formol Toxoid or Anatoxin.
D.A.T.	=	Diphtheria Antitoxin.
M.L.D.	=	Minimum Lethal Dose.

Summary.

(a) A brief survey of the history of diphtheria is given in an attempt to explain the present-day view of the epidemiology of the disease. The facts show that isolation has failed as a preventive measure. 'Latent immunisation', a natural process, is always going on in a community -- and it is suggested that artificial immunity reinforces a natural process, and runs counter to none.

(b) An account of the development of immunisation is given, showing the trend of opinion in favour of F.T. as the more efficient agent. The disadvantage of reactions is emphasized in the literature.

(c) An account is given of actual immunisation work which was carried out. A preliminary investigation has been made into the question of reactions following injection treatment.

An account of the work in several institutions where various materials are used, is given. The results have been analysed according to the reagent used, and method of employment. Alum Toxoid has not been included.

(1) The question of sensitivity to the toxoid is discussed and the avoidance of reactions considered. The use of the Moloney test is a satisfactory method. The sensitivity appears in some way connected with the development

of latent immunity and is not dependent on serum sensitisation. F.T. does not produce sensitisation to the sera used in therapy.

(2) Special points of interest are recorded.

A short history of this disease is required to give a true appreciation of the present view-point. The facts were obtained from "The Prevention of Diphtheria" Graham-Forbes[1] and "Die Diptherie seit Bretonneau"[2]. Clinical observations of diseases of the throat, suggesting diphtheria, were recorded by Hippocrates 5th Century B.C. and in the second century A.D. by Aretoeus of Cappadocia.

No great advance was made in the study of the disease until the 19th Century. There are records of "angina maligna" in Spain and Italy in the 16th and 17th Centuries. In the 18th Century records of a throat disease are found in Great Britain, but it was never differentiated from Scarlet Fever. In America Samuel Bard wrote on "throat distemper" (New York, 1771).

To a Frenchman belongs the credit of distinguishing diphtheria as a separate clinical entity. In 1821 Bretonneau published his classic "Traité de la diphthérie" giving a full clinical description of its many characters. With the coming of Pasteur it is not surprising that the bacterial origin of the disease should have awakened

interest, and in 1884 the organism was discovered by Klebs and Loeffler.

The years 1890 - 1895 marked the introduction of the sheet anchor in treatment -- anti-toxin. Behring announced his discovery at the Pediatric Congress in Berlin in 1890 and by 1895 its use had become general. The case mortality fell from 30% to below 10%, as also did the mortality rate. This was 0.26 per 1000 persons living in the decade 1891 - 1900 and 0.058 per 1000 persons living in the year 1932[3]. This fall in case mortality and mortality rate was not accompanied by a fall in the incidence of the disease. The following figures from the London Metropolitan area (M.R.C. 115) show how the incidence has not altered.

Year Group	Notification per 1000 persons living
1897 - 1903	18
1918 - 1924	19

Three great advances in the knowledge of this disease were made within the 19th Century, the clinical differentiation, the discovery of the causal organism, and the introduction of an efficient method of treatment. The case mortality and mortality rates were decreased, but as the above figures show the incidence remained stationary. The fourth great advance, with which this thesis is primarily concerned, is the artificial pro-

duction of immunity against diphtheria, and a short account of the main features of the progress of the investigation will not be out of place.

In 1903 toxin-antitoxin was used for the immunisation of horses in the production of antitoxin for therapeutic purposes by Park of New York. Four years later Theobald Smith, from experimental work in guinea-pigs, suggested the use of toxin antitoxin for human immunisation. It is interesting to note that G. Dean, writing on the Formation of Antitoxin in Nuttal & Smith's "Bacteriology of Diphtheria, 1908"[4] remarks "Active immunisation of man in the case of diphtheria has been little practised. So far as the writer has been able to determine, only one case has been recorded . . . such a heroic measure would be only justifiable in troublesome chronic cases." In this instance small doses of toxin were employed.

The above extract indicates how, in 1908 active immunisation was looked upon as an experimental method of therapy, and not as a method of prophylaxis. The means employed in preventing spread were the isolation of cases and known carriers. No theoretical arguments are required to show that isolation alone is insufficient to diminish the incidence. The explanation, given by Friedemann[5], when working in Berlin, is as follows. He

estimates that only 3% of cases of diphtheria arise from a known case or carrier -- the others being infected by unknown carriers. From investigation in certain districts of Berlin, he found that 2% of the population at any one time were carriers of virulent organisms, and that the carrier state persisted, on an average, for about 2 weeks, i.e. 100% of a population being carriers within 2 years! -- showing the impossibility of adequate isolation. Arguing from these facts, Friedemann produced his theory of "latent immunisation" arising from subclinical infections. Active immunisation can be named artificial latent immunisation.

The first attempts to immunise human beings with toxin-antitoxin were made by Behring in Berlin in 1913. In the same year Schick of Vienna introduced what was to put the whole question of immunisation on a sound basis -- an easily performed test for immunity and susceptibility to diphtheria. Although not 100% accurate a S- individual is unlikely to take diphtheria. [Discrepancies will be discussed later.] By this test it has become possible to determine the susceptibility of the population, and the immunising power of different materials.

It must be mentioned that attempts were made to immunise, as early as 1905, with the diphtheria bacillus, but it was soon realised that some form of the toxin was

the most suitable agent.

The Great War interrupted the application of the Schick test and immunisation in Europe but Park and Zingher used it on a large scale on the children in New York, and later, throughout the United States.

About 1920 testing and immunisation was commenced in this country on a large scale: it was carried out under the auspices of the Ministry of Health and many local authorities. It is worth while attempting to consider some of the results obtained by various workers. Many have been recorded. It is not proposed to quote the literature fully but to mention some results, giving indication of the trend of opinion.

The early reports deal with toxin antitoxin. O'Brien[6] in 1925, writing on Active and Passive Immunisation against common Infectious Diseases gives results of treatment with toxin-antitoxin but does not mention anatoxin. Rolleston[7] in 1926 mentions the use of anatoxin as being better than toxin-antitoxin. Feierabend[8] showed that F.T. produced a quicker immunity in animals than toxin-antitoxin. A French observer, Leviez[9], recorded that with F.T. 95 - 99% were immunised in 6 weeks, while only 80% after T.A.M. in 3 - 6 months. Kraus[10] remarks that the method is devoid of risk. Seligmann[11] in 1931 notes the following results:-

96.6% immune after F.T.

87.8% " " T.A.M.

58.9% " " Inunction with toxin.

These few extracts from a very copious literature indicate a trend of opinion in favour of anatoxin as opposed to the older T.A.M. method. Points in favour of this anatoxin seem to be that it produces a better and more rapid immunity. Another advantage is that fewer injections are necessary. The importance of this is shown by figures indicating the default rate.

Beaudet[12] in an account of immunisation in Quebec gives the following figures. The subjects were pre-school children 6 months - 6 years.

1,368 received 1st injection.

1,021 " 2nd "

802 " 3rd "

There seems to be general agreement that F.T. is a more powerful agent, but here and there emphasis is laid on the occurrence of reactions, both local and general. Ramon and Helie[13] reports slight reactions in 20 - 40% of cases, moderate in 10 - 15% and severe 1 - 5%, while Hartmann-Karplus[14] reports 83% mild, 15% fairly severe, and 2% very severe reaction.

A Chinese worker -- Lai[15] -- notes that the toxoid is much superior to T.A.M. but undesirable reactions occur. In Canada this liability was early recognised. It was

noticed that they were less frequent in children. Moloney and Fraser[16] conceived the idea of injecting diluted toxoid in an attempt to predict the likely reactors. They used a $\frac{1}{20}$ dilution of toxoid in place of the usual control Schick test. They remark that a S- reaction is presumptive evidence of immunity, but they also conclude that a +ve reaction to the F.T. is some indication that the individual is likely to be immune. Part of this thesis will be taken up with a consideration of what this reaction to F.T. is.

O'Brien, working at the Wellcome Physiological Research Laboratory is the pioneer of the use of F.T. in this country. He and Parrish[17] report the use of the Moloney test [described in detail later] noting about 5% of severe reactions in all ages up to 12 years. They mention that a definite reaction to F.T. shows induration and not mere redness of the skin and that the test is reliable, and -ves show no reactions. They also report a very rapidly produced immunity -- 4½ weeks after the 1st, of 3 injections of 1 cc. F.T.

The above references to the literature on the subject do not claim to be exhaustive, but they do indicate the problem -- to be able to use a powerful agent such as F.T. and to avoid unpleasant reactions.

The intradermal injection of a diluted toxoid can detect likely reactors. Chesney[18] of Poole

emphasizes the difficulty of technique of a skin test, especially in young children, and suggests the preliminary injection of 0.1 cc. F.T. as a "detector dose". He also notes that three injections are more efficient than two in the production of immunity. McSweeney[19] of Dublin quotes experiences in both methods. He notes that the % Moloney + rises from 7% in Age Group 0 - 2 years to 19% in 8 - 10 years. From this he remarks that the Moloney test is desirable in children under 8 years of age, when it is proposed to use potent F.T. He also used the "detector dose" method, but states that in children it is not very reliable, 5 out of 18, -ve to this detector dose, gave reactions, with temperatures up to 100.6°F. in 2 cases. He notes that the Moloney is positive in 24 hours and rapidly fades, while the Schick is at its maximum in 4 days, necessitating 2 visits. The experience in the present investigation in this matter will be noted in the accounts of the work done.

Having in view the difficulties which arise in diphtheria immunisation, this thesis will attempt to show:-

(1) a comparison between different immunising agents, especially in connection with results and reactions.

(2) a consideration of questions of immunity, etc. which may arise in this investigation.

(3) Any special feature of interest. Many have been noted. In most cases this will be remarked upon.

(4) Any conclusions which may be drawn from this work, as to the most convenient practical means of carrying out diphtheria immunisation.

The word 'practical' is of some importance, as this thesis is not intended to deal with more than this aspect of the work. The Schick Test is taken as a reliable index of immunity. There are many references in the literature to Diptheria in 'S-' individuals. Moloney and Fraser[16] report cases which were 'S-' with less than $\frac{1}{50}$ A.T. units per cc. of blood, but clinical workers are agreed that for their purpose it is a satisfactory test.

Joe[20] describes it as a 'satisfactory guide'. Parrish and O'Kell[21] agree that the 'S-' state is more or less permanent. Harries[22] in 1930 asserts that "no 'S-' person has ever had clinical diphtheria". Bousfield[23] in presenting the case for Schick testing as a routine measure describes a 'S-' state as a "training in anti-toxin production", the real basis of active immunisation. Rhoads[24] is of the opinion that impotent toxin and bad technique is the cause of 'S-' reaction in susceptible individuals. The work of McLeod, and others,[25] in Leeds helps to explain the discrepancies in the test, by their differentiation of the 'mitis' and 'gravis' type of organism. Parrish and Wright in 1935[26] report several small epidemics of diphtheria in 'S-' individuals. The epidemics occurred in a residential school, an orthopaedic

children's hospital, and a general hospital. They noted that the cases were all due to the 'gravis' type of organism. Those occurring in 'S-'s were all mild cases clinically and recovered completely without complications. (These cases were both immunised and natural 'S-'s.)

In the Orthopaedic Hospital mentioned, the carrier rate for 'gravis' organisms was 40% -- the patients here were immunised as a routine. Dudley, May and O'Flynn[27] describe an epidemic due to the 'gravis' organism in the Greenwich Hospital School. The cases were mild and often 'unrecognisable clinically'. The school consists of 900 boys and it kept at a 90% 'S-' level. They are of the opinion that immunisation prevented an outbreak of severe diphtheria, such as has recently occurred in the North of England. It need only be said that a test which indicates that a person will only suffer from a mild disease instead of a severe one, with a high mortality rate, is of great value.

One seems justified, therefore, in relying on skin tests in judging the efficacy of an immunising agent or method. In the actual employment of immunisation in a Public Health Department, toxin titration of the blood is not practicable.

Preliminary Inquiry.

As reactions appeared from all accounts in the literature to be the limiting factor in the employment of any immunising agent, especially Formol Toxoid, it was decided to try the effect of small and gradually increasing doses before attempting immunisation. The question of reaction may appear to be over-stressed, but no apology is made for this. A child who is not satisfactorily immunised has a very good chance of escaping diphtheria, but a sore arm is always prominent. It is the reactions which bring a method into disrepute.

The first trials were carried out in a fever hospital in Edinburgh on children aged up to 10 years.

Each child received the injection of F.T. into the deltoid muscle, with an ordinary syringe. The site of injection was examined after 24 hours, and any abnormality noted, whether local or general. The reactions were classified as follows:-

(1) Slight local -- there was slight redness and swelling at the site of injection with perhaps some stiffness but no real pain. The patient suffered no inconvenience of any kind.

(2) Moderate local -- in this case the whole upper arm was swollen and painful. Movement was

difficult and definite inconvenience was caused, but the patient never felt ill.

(3) General reaction -- in this case there was pyrexia and malaise within 24 hours. It was noticed that there never was a general reaction without a local one.

Children suffering from diseases other than diphtheria were chosen and received very small doses -- 0.1 cc. of F.T., an extremely cautious start. 57 cases received this small dose, and there was absolutely no reaction of any kind. Ten days later each child received 0.5 cc., with only a very mild reaction in a single case. The above results indicated that it was possible to commence actual immunisation of non-diphtheritic cases with Formol Toxoid. This was also limited to children under 10 years, unless it was specially desired. Permission was obtained from the parents in all cases.

The first amounts were three injections of 0.5 cc., 1.0 cc. and 1.0 cc. at 10 days interval. When it was found that 1.0 cc. caused little disturbance the first injection was raised to 1.0 cc. In all, over 300 children were treated in this way. All these cases were personally inspected after 24 hours and the reactions noted. In addition they were under the observation of the nursing staff.

No severe reactions were seen: the actual results

are expressed in the Table.

0.5 c.c.

Age	0 - 5	5 - 10	10 - 15
No. treated	74	118	19
Reactions	4 mod. local	10 slight 4 mod. local	4 mild 1 mod. local 1 general

1.0 c.c.

No. treated	66	94	17
Reactions	5 mild 1 mod. local 2 general	11 slight 6 mod. local	8 slight 2 general

Daily inspection was then discontinued and over 1000 cases were immunised with only an occasional general reaction.

An attempt was made to appreciate the parents' viewpoint, and it was decided that:-

(1) no parent would object to a slight local reaction.

(2) If warned beforehand of such a possibility, no objection would be raised to a moderate local reaction

(3) Even a few general reactions would bring the method into disrepute.

The results from this preliminary inquiry suggest that, while F.T. can and does cause severe reactions,

their frequency is greatly exaggerated.

An interesting point arose. When it was first decided to immunise non-diphtheria cases, Schick tests were performed on all children. It was found that, amongst the scarlet fever cases, almost all were S-, an impossible result. The explanation of this was that routine serum treatment was being used for scarlet fever, and the natural Diphtheria Anti-toxin in horses' serum conferred a temporary passive immunity on these children.

These preliminary observations were therefore of value only from the point of view of reactions, as no record could be obtained of the Schick state of the subject.

Record of Work Carried Out.

A description will now be given of the work carried out, and results obtained, in the various institutions, but before this is done, mention must be made of the technique used.

The Schick test was performed in the usual manner, 0.2 c.c. of diluted toxin, containing $\frac{1}{50}$ M.L.D. were injected intradermally into the skin of the forearm and, when a control test was performed, heated toxin was similarly injected into the other arm.

The Moloney test is not standardised as is the Schick test. In the present series Formol Toxoid was diluted 1:20 and 0.2 cc. injected intradermally into the forearm. When positive, the maximum intensity developed after 24 hours.

The types of results obtained were

1) completely -ve

2) a patch of redness similar to the Schick reaction

3) Definite induration, usually about 2 cms. in diameter, surrounding the site of injection. On rare occasions this induration involved the whole forearm.

The interpretation of these various results had to be decided, and one must anticipate the information recorded in a later section. Three conclusions were drawn:-

(1) a completely negative result indicated that F.T. could be safely given -- the maximum dose, in the present cases, being 1.5 cc. This was shewn by the almost complete absence of any reaction in -ve cases.

(2) mere redness could be regarded as a negative result and full doses could be safely given.

(3) the criterion of a "Moloney +" is induration and an injection of F.T. would cause symptoms. The exact severity of probable symptoms was difficult to determine, as the aim was to avoid

them. It is sufficient to note that there was always some malaise when toxoid was given to a case showing in induration, and for practical purposes, none followed an injection of F.T. into a case showing no induration.

These criteria, of course, only hold for a dilution of 1:20 of the toxoid. Other workers -- such as McSweeney[19] -- used more dilute solutions of 1:200, where, presumably, redness indicated a positive reaction. He reports that the test had to be read not later than 48 hours, necessitating a special visit of inspection. The use of the stronger solution in this investigation made reading possible after 8 days, although admittedly this was difficult. This alone is an adequate reason for the use of the stronger solution.

In the records of work it has been assumed that, in the Moloney test, induration indicates that a person is sensitive to F.T.

Pontoon Street, Edinburgh.

The first institution to be immunised in this investigation was a hostel for youths of the errand boy type. The boys come from all parts of the country -- both rural and urban districts. The home is situated in a slum area although the buildings themselves are of good construction with very satisfactory ventilation. There

is no suspicion of overcrowding.

The ages vary from 12 - 20 years. While the boys live in the hostel they mix freely with the general population at work, in evening classes, etc. They are under discipline inasmuch that they must keep themselves clean and any case of sickness is immediately reported. This home had been immunised about 18 months previously, and this had been so successful in preventing cases, that the managers themselves asked that it should be repeated, especially as several cases had occurred and the population is continually changing.

It was decided to use Formol Toxoid wherever possible, in spite of the fact that subjects were over 10 years of age. The attitude to immunisation was not in any way hostile, but could be defined as "suspicious", and one was therefore particularly anxious to avoid any reactions likely to bring the method into disrepute.

The boys were all Schick tested (including a control test) and they were also Moloney tested. The Moloney test was read in 24 hours, the Schick test after 1 week -- the week's interval being chosen in the case of the Schick test as Sunday was the most convenient day for the inmates to be collected together. The special visit for the reading of the Moloney test is a disadvantage of this method. As however this was the first time

the Moloney test was being "used" as an integral part of the method, it was not thought to be wasted time to read it at its maximum intensity.

The cases were divided into 3 groups:-

(1) S-

(2) S+ M-

(3) S+ M+

S- cases required no further treatment.

Of the S+ cases, Moloney -ves received Formol Toxoid, 0.5 cc., 1.0 cc. and 1.0 cc. at 14 days intervals. Moloney +ves received Toxoid Anti-toxin Mixture 0.5 cc., 1.0 cc. and 1.0 cc. also at 14 days intervals. By this means it was hoped to reduce any risk of reaction to a minimum. It also served as a means of comparing the two reagents.

After each injection the patients were examined in 24 hours. In both groups Schick and Moloney tests were done 2 weeks and 6 weeks after the final injections -- 6 and 10 weeks after the 1st injection.

Results.

Total Tested	S+		PS	M+
	M+	M-		
45	7	10	1	20

Results of Treatment.

	M- receiving F.T.	M+ receiving T.A.M.
S+ cases immunised	10	7
S- 2 weeks after last injection	10	3
S- 6 weeks after last injection	10	6

No severe reactions were noted in any case. A few boys complained of slight local pain and stiffness, which caused no inconvenience. No work was lost.

On inquiry 6 months after immunisation no case of diphtheria had occurred.

One case aged 16 showed an interesting feature. There was an intense reddened area about 5 cms. in diameter at the site of the Schick test and control. Engorged lymphatics ran up the arm from these areas as far as the elbow. A faint scarlatinal form rash developed on the front of the chest; but there was nothing else suggesting scarlet fever, the fauces being perfectly healthy and the boy feeling well. Unfortunately it was not possible to do a Schultz- Charlton Reaction in this case and the condition cleared up in about 12 hours. The Moloney Reaction was "strong +ve". The boy was successfully immunised with T.A.M. without any reactions.

The experience in this institution indicates:-

(1) The superiority of F.T. over T.A.M. -- especially in the rapidity of immunisation.

(2) The possibility of avoidance of reactions.

(3) The marked sensitiveness some cases show to the Proteins of the Schick toxin.

Note.

The 'S' results were all classified as mild, average and severe, but these are not mentioned in each single account, but are incorporated in the Summary, as is also the change in the Moloney reaction.

Gogar Burn Institution.

This institution is for mental defectives of all grades from the City of Edinburgh, and is situated on the outskirts of the town. It has only recently been built and is on modern hygienic principles. There are about 400 inmates, the ages varying from under 5 years to the 50s and 60s. These inmates are all from urban areas.

The staff are drawn from all parts of Scotland, rural and urban. They were too few to have any statistical significance but strangely most S +ves among them came from the Lanarkshire mining areas, while the S -ves came mainly from the Highlands.

For the past year or so diphtheria had become "endemic" and at times small epidemics occurred. The

isolation of cases and known carriers was not effective in stamping out the disease, so it was decided to immunise the inmates.

Relatives of mental defectives are notoriously "difficult", and before any patient could be tested and immunised permission had to be obtained. Later permission was given by the Board of Control to treat patients who were without relatives and finally a third group, consisting of persons who missed the previous Schicking, etc. for various reasons, was tested.

The usual technique was employed for the Schick, Schick Control and Moloney test. As the Institution Authorities wished the staff and patients to be immunised against Scarlet Fever as well, Dick tests and controls were done simultaneously with the other tests. The Moloney and Dick reactions were read after 24 hours -- the Schick reactions after 4 days. As the Moloney test was still in the nature of an experiment, it was omitted in the testing of the Staff. Where necessary they were immunised with T.A.M.

"S-" cases require no further consideration. The "S+" cases were divided into the following groups:-

(1) M+ and D+

(2) M+ and D-

(3) M- and D+

(4) M- and D-

In order to keep the number of injections to a minimum, the two materials -- against Diphtheria and Scarlet Fever -- were given at the same time.

As there were no experiments in connection with the Scarlet Fever immunisation a well tried scheme of dosage was employed -- 4 injections of 500, 1,000, 5,000 and 20,000 skin test doses. There was no point therefore in cutting down the number of antidiphtheria injections in the 'D+' groups, so they were given 0.5 cc. 1.0 cc., 1.0 cc. of T.A.M. or F.T. according to their Moloney state. Of those not requiring Scarlet Fever immunisation, the "M+"s received the 3 doses of T.A.M. 0.5, 1.0 and 1.0 cc. The 'M-'s received only 2 doses this time of 1.0 cc. and 1.5 cc. F.T.

The injections were given at intervals of 10 days. All cases were reschicked 3 weeks after the 1st injection, i.e. about 6 weeks after the commencement of treatment.

The Staff of the Institution were similarly "Schick" and "Dick" tested, and they (the 'S +ves') received T.A.M. 0.5, 1.0 cc. and 1.0 cc. and the same four doses of Scarlet Fever prophylactic. They were retested 4 weeks after the final dose.

A second batch was tested about 4 months after the 1st batch, actually in 2 batches at 20 days interval. They received similar treatment, with the exception that

the 1st dose of F.T. was reduced to 0.5 cc., a total of 2 cc. being given.

The following tables give the results of testing and immunisation.

Staff.

Total Tested	S+	PS	D+	PS
52	19	1	3	-

Results of immunisation with 0.5, 1.0 and 1.0 cc. T.A.M.

No. Immunised (S+) 13

"S-" in 4 weeks 10

No Moloney tests were performed on the Staff and only T.A.M. was used. The reactions are noted below, and were much more marked here than in the case of the patients, where the method was determined by the Moloney reactions -- This in spite of the use of T.A.M. alone. This fact is recorded without comment as (1) the numbers are small and (2) a dulled intelligence of a mental defective would not notice a reaction as easily as a person with normal intellect.

Reactions.

No. immunised	General Reactions	Local Reactions.
13	6*	2

* one special type of reaction occurred which is noted later.

1st Batch among the patients.

Total tested	S+		PS	D+	Pseudo Reactions	M+
	M-	M+				
90	16	16	4	25	1	33

Results are tabulated in the groups according to the material and amount used.

(1) Received 0.5, 1.0, 1.0 cc. T.A.M.

No. treated	6	Reactions
S-ve 2 weeks after last injection	3	3 local reactions
S-ve 10 weeks after last injection	3	

(2) Received F.T. 0.5, 1.0 & 1.0 cc. (also immunised against Scarlet Fever).

No. treated	8	Reactions
S- 2 weeks after . .	8	3 local 1 mild general
S- 10 weeks after . .	8	

(3) Received 1.0 and 1.5 cc. F.T.

No. treated	8	Reactions
S- 2 days after . .	7	1 local 2 general
S- 10 weeks after . .	8	

Reactions are noted in the tables. In no case did they cause more than slight inconvenience. In the case of one general reaction the patient was confined to bed for about a day.

2nd Batch among the patients.

Total Tested	S+		PS	D+	Pseudo Reactions	M+
	M-	M+				
38	3	4	-	6	-	10

Immunisation

'M-ve' received 0.5 and 1.5 cc. F.T.

and 'M+ve' " 0.5, 1.0 cc. & 1.0 cc. T.A.M.

Owing to my leaving Edinburgh I was not able to retest these cases personally, but I understand that they were satisfactorily immunised.

As the number of cases is small no special exphasis is put on the results beyond their value as 'impressions'. It has been stated that diphtheria and scarlet fever immunisation interfere with one another. In the following table the results of immunisation against diphtheria in 'D+' and 'D-' cases are compared.

	D+ cases	D- cases
Not treated	11	11
S- 10 weeks after	10	8

The treatment was the same in both groups, except that the 'M-' cases in the 'D-' group received 1 or 1.5 cc. F.T. as compared with 0.5, 1.0, 1.0 cc. in the 'D+' group.

T.A.M. doses were the same in all cases.

No. treated with S.F. Prophylactic	25
D- after 10 weeks	17

These figures do not suggest that there is any interference.

The results indicate,

(1) Reactions can be avoided with F.T., which is a more efficient and rapid immunising agent than T.A.M.

(2) Scarlet Fever and Diphtheria Prophylactics may be given at same time.

A peculiar Reaction to Injections of T.A.M.

A nurse aged 24 was tested with both the "Schick" and "Dick" tests. She was 'D-' but 'S+' the reaction being of moderate intensity. She had had an attack of diphtheria 14 years ago. The Moloney test was not done and she was treated with 3 injections of T.A.M. of 0.5, 1.0 cc. and 1.0 cc. There was a slight local and general reaction after each injection.

The unusual feature was the appearance of herpes labialis after each injection. The nurse felt feverish about 12 hours after injection. The herpes appeared in 24 hours and she then felt quite well. The herpes pursued a normal course.

No reference could be found to this type of

reaction in literature on diphtheria but it makes an observation of Alderhoff[28] particularly interesting. He is writing of diphtheria immunisation in Holland and notes that Anatoxin produces a strong reaction, but is a most effective immunising agent. He is of the opinion that any reaction is liable to produce an encephalitis of the post-vaccinal type, which is unknown in younger children. He naturally advises early inoculation to prevent this.

'Schick +' reactors among patients who have had Diphtheria.

Of the cases tested 10 were known to have had diphtheria previously, the time varying from about 4 months to 14 years. It is interesting to note that 8 of these were "S+" indicating that second attacks will not be too uncommon.

Swab Results.

The difficulty of interpreting swab results is well shown in 2 cases. No record of virulence could be obtained.

They both gave a '+' swab result for 6 weeks, but were clinically free from diphtheria. At the end of this period one was admitted to hospital as a definite case. Finally when tested, 8 months after the above noted swab examinations both were 'S+'.

The intermittence of the carrier-state is shown by 2 cases

(1) + 23 July
- 27 "
+ 1 August
- 10 "

(2) - 15 July
+ (17 "
(
+ (27 "
(
+ (1 August
- 10 August

Mayfield House Children's Home, Edinburgh.

This institution is a home for the children of personnel of the Royal Navy. They are drawn from all parts of the country and ages vary from 4 to 16 years. Children of both sexes are admitted. The actual premises consist of a modernised mansion.

The children form a semi-isolated community; they live permanently in the home but mix freely with other children in attending school, church, etc. In these circumstances testing and immunisation is particularly easy.

There is a constant change, there being an annual admission rate of 10. The children in the institution were immunised in 1926 and only a few

sporadic cases of diphtheria occurred until about 6 months before the present testing. From October 1933 until April 1934 out of a population of 40, 10 cases of definite diphtheria occurred and 2 carriers were discovered.

The authorities of the home decided to have immunisation carried out, but before proceeding permission slips were sent out to the parents or guardians. It is interesting to note that there was only 1 refusal, affecting 2 children and this was because they had been tested and immunised a few months previously. This illustrates that among parents there is no real antagonism to immunisation -- that there is in fact as, Benson describes it[29], "a latent demand".

The same routine was used as in the previous experience. All children were Schick-tested (including a control) and Moloney tested. Readings were taken in 24 hours and in 5 days. In this instance the extra visit for the Moloney reaction was most conveniently carried out.

Formol Toxoid was used throughout without reference to the Moloney state, and 2.5 cc. were used for each patient, but this time in 2 doses -- the first of 1 cc. and the 2nd of 1.5 cc. at 10 days interval. The injections were given in the deltoid muscle.

This may appear somewhat unscrupulous, but toxoid was used even when a severe reaction could be predicted. In a few cases this was very severe. Notes of the reactions are recorded. The cases were all re-schicked and Moloney-tested 14 days after the final injection.

Result.

Total tested	S+		PS	M+
	M+	M-		
36	3	13	2	17

Results of Treatment.

		Reactions	
S+ cases treated	16*	M+	5 severe
S- 14 days after . .	14	M-	1 moderate
S- 6 weeks after . .	15		

*Two of the above noted cases gave such a severe reaction with 1.0 cc. F.T. that they were given 0.5 cc. F.T. as a second injection, a total of 1.5 cc. Both were Schick +. One became Schick -ve within 14 days, the other case within 6 weeks.

The results show:- (1) a successful immunisation with F.T.

(2) a warning note -- sensitive children can be made really ill by the uncontrolled administration of F.T. Even a few such reactions would condemn the method among the public.

Reactions.

This institution gave the best illustrations of severe reactions. Two are recorded to emphasize the importance of avoiding them.

(1) Female aged 14+ Moloney +

Reaction after 12 hours, Headache, vomiting.

Temperature of 101 for 3 days.

(2) Male aged 9. Moloney +.

Temperature 101 in 12 hours

102 after 30 hours.

Headache, vomiting; arm greatly swollen.

Features of Special Interest.

'S+' reactors amongst cases of diphtheria.

Seven of the cases tested were known to have had diphtheria and 2 of them remained "S+".

Peculiar Reaction to the Moloney Test.

One case, a female aged 12 years gave a '+ve' Moloney reaction, the indurated area being about 1.5 cm. in diameter. Surrounding this area was a ring of small pin-point peticheal haemorrhages about 1 cm. wide. The patient was "S-" and showed no other abnormality.

Deaf & Dumb School, Edinburgh.

This institution is a residential school for deaf and dumb children. While not leading a completely monastic existence, they do not mix freely with the

general population. The children are allowed to return to their homes at holiday times and are drawn from all parts of Scotland. The ages vary from about 4 years to 16 years.

The home was not Schick-tested before treatment. All children received 3 injections at 10 days interval, each dose consisting of 1 cc. of Formol Toxoid. This immunisation was not done personally. Reactions were not being definitely studied, but the matron reported there was no "special trouble".

The re-Schicking was performed 4 months after treatment. Any S+ were again tested 2 months later. Control tests were also done. The children were Moloney tested. (The Moloney results were of slight significance and are not recorded separately here, but are incorporated in the general summary.)

No. treated	68
S- after 4 months	60
S- after 6 months	65

The results show a successful immunisation with 3 injections of F.T. This, of course, is not making use of one of the principal advantages of the toxoid -- i.e. the capacity for immunisation in 2 injections.

Still an immunisation rate of 93% in 6 months is very satisfactory.

The above institutions are all situated within or near the City of Edinburgh and it was comparatively easy to carry out frequent visits for the recording of reactions, Moloney results and the like. The following four institutions are situated within the County of Somerset, and the work was carried out as part of the work of The Public Health Department. From reasons of distance alone, therefore, it was of importance to reduce the number of visits to a minimum and the observation of reactions was left to the Nursing Staff. The experience gained, therefore, is of great importance, as showing routine immunisation in action.

In the Edinburgh Institutions it was possible to re-Schick at intervals after the treatment -- and in one instance -- the effect of simultaneous Scarlet Fever immunisation could be studied. It was therefore thought to be worth while to analyse the results in each institution. This has not been done in the case of the Somerset Institutions -- the results are incorporated in the final tables. They may be briefly referred to, in order to emphasize some point, in the account of each individual institution.

West End House, Shepton Mallet.

This is an institution for female mental defectives.

All patients were over 15 years of age. The cases were Schick-tested (together with a control). The Moloney tests were read after 24 hours and the Schick tests after 5 days. As previously, induration was taken as the criterion of a positive Moloney. Mere redness was neglected and has been included among the negative reactions in the tables of results.

In this institution, the staff were not Moloney tested, and received a dose of T.A.F. if they were S+. It was considered better not to use Formol Toxoid on them as there was still doubt of the severity of the reactions. This institution presented no special features and no pseudo reactions occurred. No visits of inspection were made after the 2 injections, but the nursing staff were warned what they might expect and they reported no reactions.

It was possible to retest 37 cases 16 weeks after treatment. The T.A.F. cases gave an immunisation rate of 60% while F.T. cases were over 90%. Reduced doses were given to M+s and those who showed reactions after the 1st dose.

Cambridge House, Long Ashton.

This institution is a home for low grade mental defectives, drawn from various parts of Somerset. The ages vary in this institution from about 15 upwards, most

of the inmates being adults, and many having lived there most of their lives. There were 2 children about 7 years old.

A somewhat different procedure was adopted. At first, all cases, both Staff and inmates, were Schick-tested, controls being performed. No Moloney tests were carried out; the 'S+' reactors amongst the staff were treated with T.A.F.

In place of the Moloney test a "detector dose" as first suggested by Chesney of Poole was employed to determine sensitive individuals. Instead of receiving a first dose of 1.0 cc., each case received 0.1 cc. of the F.T. intramuscularly into the deltoid. Reactions both local and general were noted. Where there was no reaction a full dose was given 3 weeks later as a second injection. The full dose was also given where there was merely a mild local reaction. Two cases which developed a general reaction were given 1 cc. T.A.F. as a second injection.

It is interesting to note that in only one case was there a reaction after the injection of 1 cc. of F.T. when there was no reaction after 0.1 cc. showing that this is quite a satisfactory means of detecting reactors.

Reactions seem to be due to some qualitative rather than quantitative factor.

Results.

Total Tested	S+	Pseudo
83 patients	38	10
9 Staff	5	1

The reactions to the detector dose, and the subsequent doses given are shewn below.

	"No Reaction"	"Local Reaction"	"General Reaction"
No. of cases	32	4	2
Subsequent doses with any reaction	1.0 cc. F.T. 1 local reaction	1.0 cc. F.T. 4 local reactions	1.0 cc. T.A.F. 2 general reactions.

Cambridge House Staff.

These cases received T.A.F.

1 cc. as 1st injection	2nd injection	
5	1 general reaction 1 local " 1 local " 2 no reactions	0.5 cc. T.A.F. refused further treatment. 1.0 cc. F.T. 1.0 cc. T.A.F.

The results are included in the final summary.

Briefly only 55% of the cases were satisfactorily immunised when retested 4 months after the last injection.

Yatton Hall, Somerset.

This is also an institution for the low-grade mental defective, but belonging to the younger age groups. The bulk of the cases were between the ages of 10 and 15 years, only very few being over this. There were the usual administrative staff.

All cases were Schick-tested (with control) and as before the staff were treated with T.A.F. The detector dose method was again employed, but 0.2 cc. F.T. was given instead of 0.1 as in Cambridge House. Of the 41 cases who gave no reaction with 0.2 cc. only 3 gave a reaction with 1.0 cc., showing its value as a preliminary test. Re-Schicking was carried out after 4 months and 70% were satisfactorily immunised.

Results.

Ages	No. tested	S+	Pseudo
0 - 10	11*	10	-
10 - 15	29	27	-
15+	9	8	1
Staff	9	6	1
Total	58	51	2

* Includes 2 children from Cambridge House, included for convenience.

The following table shows reactions to a "detector" dose of 0.2 cc. with the subsequent dosages.

	No Reaction	Local Reaction	General Reaction
No. of cases	41	1	-
Subsequent doses with any reaction	1.0 cc. F.T. 3 slight local reactions	1.0 cc. F.T. -	-

Sandhill Park, near Taunton, Somerset.

This institution is a residential school for educable defective children, including some young adults. The buildings consist of an old country mansion -- the administrative block, and modern ward blocks. It is situated about 4 miles from Taunton. Cases are drawn from the whole of the county of Somerset -- the majority therefore coming from Rural Districts. The ages vary from about 5 - 20 years. The institution was immunised with Toxin Antitoxin Mixture in 1929.

Previous experience has shown that a pseudo-reaction was extremely rare occurring in less than 1% of cases, and so no control tests were performed. This reduced the work by one third and what is more important eliminated an unnecessary pain. Moloney tests were performed on all cases and the doses of the toxoid were determined by the results.

Reasons of distance made it impossible for the Moloney test to be read at its period of maximum intensity i.e. after 24 hours. A 1:20 dilution of the Toxoid was used for the test, and as this was a much stronger solution than is generally advocated, it was hoped that any reaction would persist and be readable after a week. The same criterion of a positive reaction was chosen, i.e. induration. In many cases the redness of the reaction had faded, but induration could be detected on fine palpation.

It is necessary to be very familiar with this test when reading it after the lapse of a week. In many cases induration was hard to detect, and one felt no confidence in the result. One case must be mentioned in particular. The nursing staff reported that there was extreme induration and redness 24 hours after injection, but there was no trace after 7 days. It is worth noting that this case was S-.

However the answer to the question of reading after a week is found in the results. The nursing staff were asked to be on the look-out and they reported no troublesome reactions. Cases showing induration received the smaller doses, while the others received the full dose. This is, for practical purposes, a successful reading of the test, thus showing that it was possible to pick out the reactors after one week provided one was familiar with the appearance of the arm.

Results.

Total Tested	S+		M+
	M+	M-	
74	9	77	29

Injections. F.T. used in all cases.

	M-	M+
No. of cases	87	11
Dosage of 1st injection	1.0 cc. F.T.	7 cases received 0.5 cc. F.T. 4 cases received 0.2 cc. F.T.

All cases, no matter what their Moloney state were given 1.0 cc. F.T. as a second injection. It was not possible to re-examine the cases but some were reported to have suffered from a malaise the day following treatment.

The 1st injection of Toxoid was given when the Schick test was read. The 2nd was given after an interval of 3 weeks (in 6 cases 3½ weeks). Reschicking was performed 3 months after the 1st injection. An immunisation rate of 92% was obtained.

Sandhill Park is interesting as it was the last institution to be treated and the details of method employed were fruits of previous experience. The number of visits was cut down to a minimum by:-

(1) the elimination of the Schick control.

(2) the reading of Schick and Moloney test on the same visit. (This was successful although the reading of the Moloney was rather difficult.)

(3) the treatment with 2 injections of F.T. The patients were completely treated in 4 visits, including a final Schick test.

There was an absence of reactions and the % of immunised was satisfactorily obtained.

Results of Immunisation.

This is a summary of the results recorded under the various accounts. The dosages are classified as:-

(1) Standard Dosages, i.e. when a case receives a definite amount of the immunising agent, in doses planned before commencing the treatment.

(2) Special Dosages - where the full dose of Toxoid was contra-indicated on account of a strong +ve Moloney reaction or severe general reaction following a previous injection of F.T. The dosage was decided individually in each case. The question of reactions arose in the case of F.T., and modification took the form of reduced dosages or employment of some other preparation. The 3 agents used in this investigation were:-

a) Formol Toxoid

b) Toxoid Anti-toxin Mixture.

c) Toxoid Antitoxin floccules.

Results are shown (1) according to material used

(2) " " dosage.

Standard Dosage.

Material used	Dosage in cc.	No. treated	No. re Schicked	No. Schick-+ve	% immunised	Remarks
F.T.	.5,1.0 & 1.0 cc.	18	18	-	100	Moloney -ves
	1.0,1.0 & 1.0 cc.	68	68	8	* 82	Unselected
	1.0 & 1.5	27	25	2	92	Unselected
	1.0 & 1.0	111	106	8	92.5	Moloney Method
	.1 & 1.0 cc.	40	38	17	50.2	"Detector Dose" Method
	.2 & 1.0 cc.	42	40	11	72.5	
T.A.F.	1.0 & 1.5 cc.	5	5	2	60	Staff of some institutions & M+s
	1.0 & 1.0 cc.	10	6	2	66	
T.A.M.	.5,1.0 & 1.0	34	27	10	63	Moloney +ve mainly

* This group, consisting of children up to 12 years -- more than half from 3 - 5 years -- did not have a preliminary 'S' test. 7 months after treatment 96% were S -ve.

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* This group, consisting of children up to 12 years -- more than half from 3 - 5 years -- did not have a preliminary 'S' test. 7 months after treatment 96% were S -ve.

Special Dosages.

Material & Dosage	No. Treated	No. re-Schicked	Remaining +ve	% immunised	Remarks
1.0 cc. & 0.5 cc.F.T.	9	9	1	90%	These are cases given "Special doses" in the Moloney Method
1.0 cc. & 0.2 cc.F.T.	6	6	-		
1.0 cc. & 0.1 cc.F.T.	3	3	-		
1.0 cc.F.T. & 0.5 T.A.F.	2	2	-		
0.1 cc.F.T. & 0.5 T.A.F.	1	1	1		
0.1 cc.F.T. & 1.0 cc. T.A.F.	2	1	1	-	Detector Dose Method

The numbers in each group are not very large, and too much stress cannot be placed on the figures. A percentage of over 85 immunised can be taken as satisfactory, while materials and methods giving 70% successful results cannot be considered sufficiently reliable.

Formol Toxoid.

This was administered by 2 methods:-

(1) Moloney Test - in this method at least 2 doses were given -- a minimum of 2.0 cc. F.T.

(2) Detector Dose - where the first dose was 0.1 or 0.2 cc., followed by a 2nd dose of 1 cc.

These methods are described in the individual accounts.

The results show:-

(1) an un-satisfactory % (70% recorded) of immunisation is obtained with 1.1 or 1.2 cc. F.T.

(2) 2 injections, of 1 cc. each, give a satisfactory % of 85 and over.

Strangely enough the largest doses -- 3 injections of 1 cc. gave the poorest result! The interval between first and final injections in all cases was never less than 3 weeks.

T.A.F. or T.A.M. were given under the same conditions as F.T. and with the same or higher dosages, the results being unsatisfactory.

Rapidity of Immunisation.

25 F.T. cases were tested 14 days after the final injection. Without giving detailed figures, it is sufficient to say, that 24 were immune in 14 days; only 1 case took longer. The immunity after T.A.M. was slower, only about 50% of those who finally became immune being Schick negative in 2 weeks.

The following conclusions can be drawn:-

(1) F.T. is the most satisfactory immunising agent.

(2) it produces this immunity rapidly within 2 weeks

(3) 2 injections of 1 cc. at 3 weeks interval are as satisfactory as more frequent injections and larger doses

(4) less than 2 cc. of the Toxoid does not give a satisfactory result

(5) where, on account of severe reactions, the full dose cannot be given, satisfactory results can be obtained by modifying the dose of F.T. -- there is no need to employ any of the other materials.

The superiority of F.T. over T.A.M. or T.A.F. is more marked than the figures indicate, as the T.A.M. and T.A.F. cases were largely drawn from 'M+' reactors, who on the whole give a less pronounced 'S+' reaction than negative reactors.

Another factor of importance is the interval between treatment and re-Schicking. This is not analysed in the tables as it would mean excessive division into groups. The time interval varies from 3 - 6 months. In all cases it is longer in the groups giving a low % of immunisation. In no instance did a case negative 3 months after treatment turn positive after a further 3 months.

The reactions noticed are recorded under each separate account. It is impossible to summarise them as the way in which they were determined varied greatly. Sometimes special inspections were made after 24 hours; at other times the staff of the institution noted any

reaction. It is sufficient to say that these caused no real trouble. The only severe ones occurred when F.T. was given in full doses to a known '+' Moloney to see the effect.

The two methods of discovering likely reactors seemed to be efficient for this purpose. One can be sure of the Moloney test method. "Detector" doses also seem reliable, but this was only used in 2 institutions, in one of which the population consisted of children -- not sufficient material to be dogmatic. McSweeney[19] remarks that he has found this method unreliable.

The present experience shows that a detector dose, followed by one full dose, does not give a satisfactory immunisation rate. Two full doses of 1 cc. are required. The number of visits is, therefore, not reduced by this method as compared with the Moloney method. The Moloney test is a skin test and there is no risk of anything more than a purely skin reaction at the site of injection. A detector dose carries with it a very appreciable risk of a general malaise. And for satisfactory immunisation 2 further doses are required.

It has been said that a skin test is difficult to perform on children and that it is much easier to give an intra-muscular injection. In the present experience the technique of skin tests was not found too complicated for use in children, and caused no real

Diagram shewing percentage of "S+" Reactors
at various Age Groups [Combined Results]

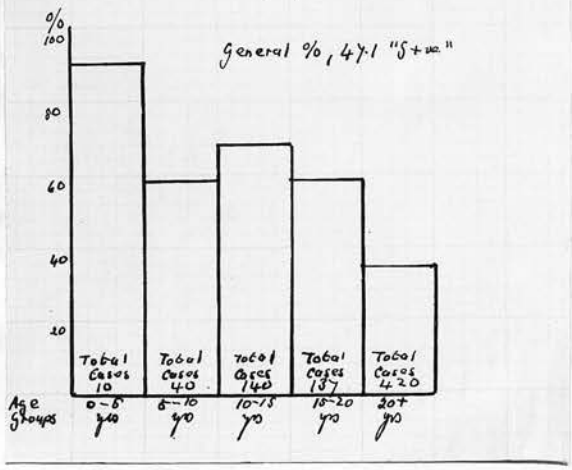
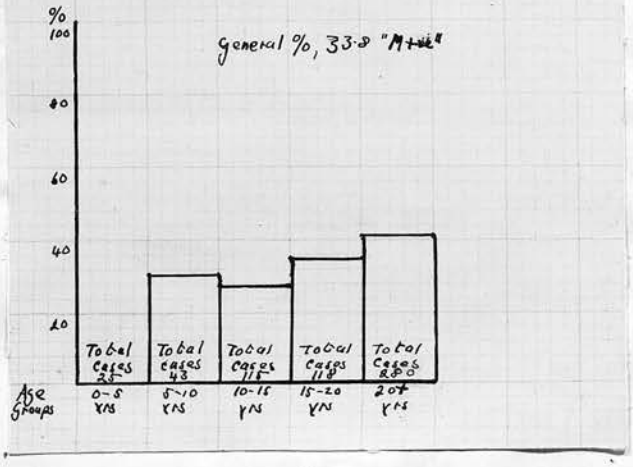


Diagram shewing percentage of "M+" Reactors
at various Age Groups [Combined Results]



trouble. The greater safety of the Moloney test more than outweighs the benefits of the easier technique of "detector doses". In addition the Moloney test reading is objective and not subjective. The present evidence, therefore, is in favour of the Moloney test method.

The Moloney Test.

Time of Maximum Intensity.

The 'Moloney' test is at its maximum at 24 hours, and rapidly fades although it can be detected at the end of a week. In one case there were a few peticheal haemorrhages forming a ring round the site of injection. It would seem that the 'substance' had an effect on the vaso-motor mechanism causing dilatation of vessels and oozing of serum. This is the usual reaction, but in certain sensitive individuals, the actual capillary wall is damaged and corpuscles escape.

Effect of age.

The tests were performed on persons of all ages, and the % varies. The results are shown diagramatically being expressed as %s in 5 year groups. It was not thought to be advisable to subdivide the groups any further.

It so happened that the work recorded in this thesis was carried out in 2 localities essentially different.

Diagram shewing percentage of Schick "+ve" Reactors at different ages

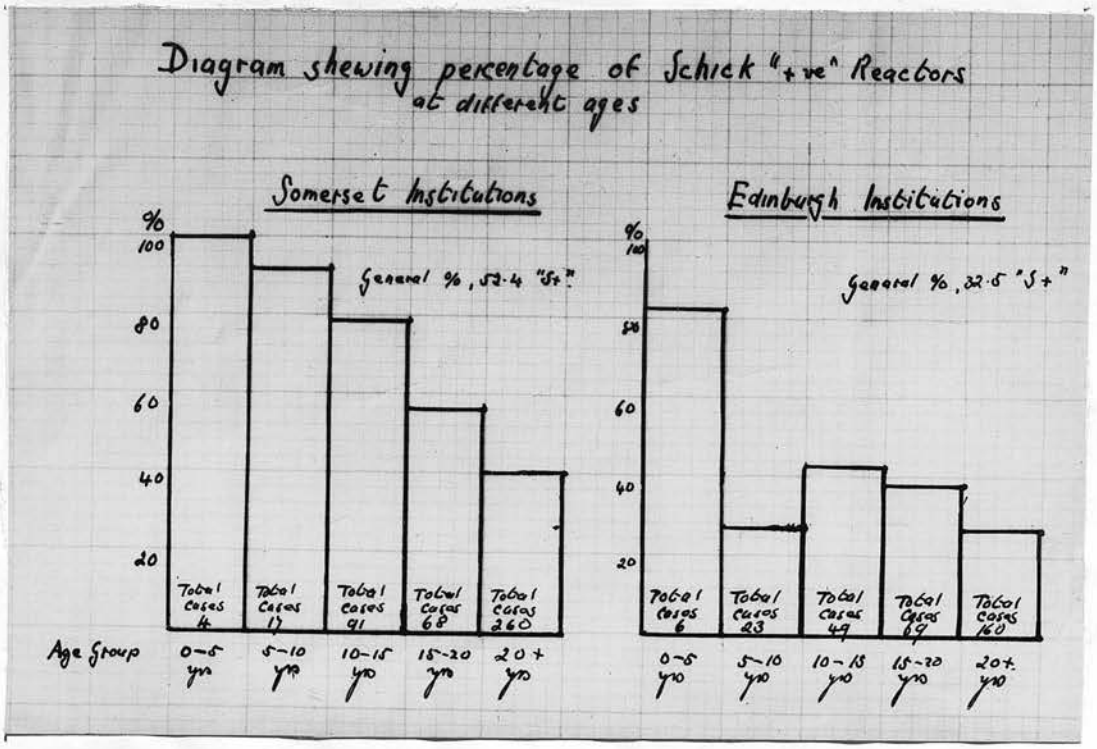
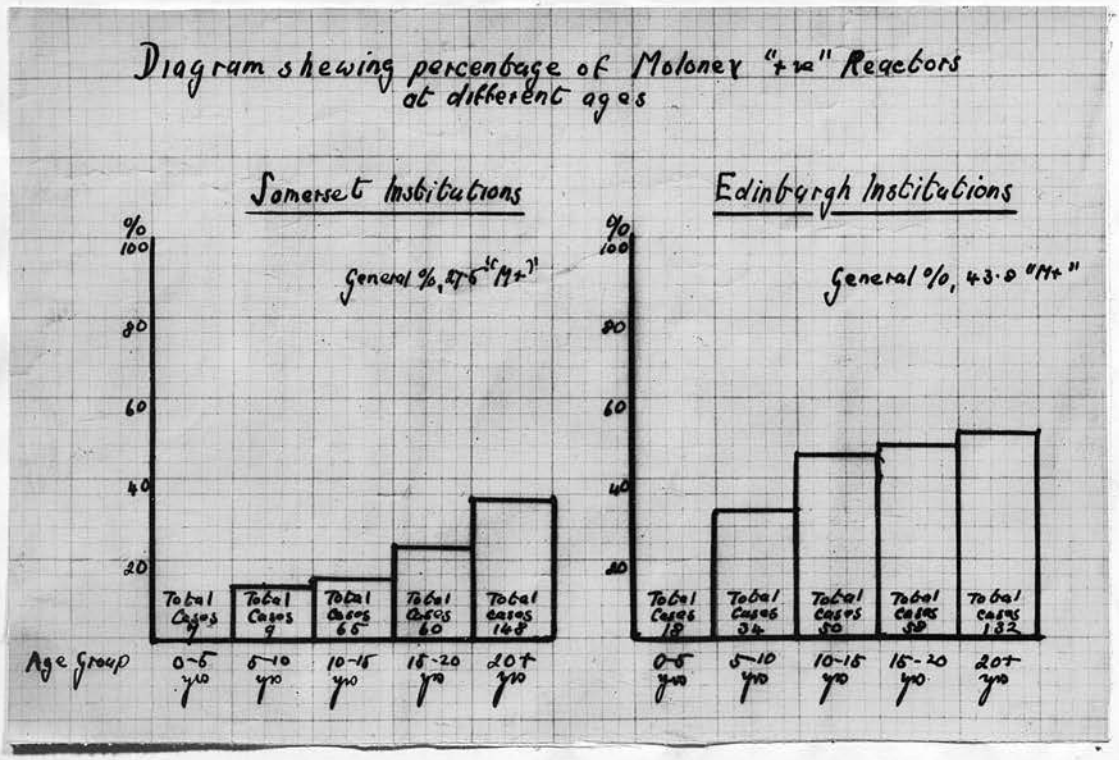


Diagram shewing percentage of Moloney "+ve" Reactors at different ages



In addition to combining the results in one diagram two diagrams have been prepared, one from cases drawn from a city -- Edinburgh, and the other from cases coming from a district mainly rural -- the County of Somerset. The persons tested in each instance were of similar type -- mainly institutional inmates, with some normal school children.

Both diagrams show the same phenomenon with reference to the age -- a steady increase of M+ reactors in the increasing age groups.

Between the 2 types of communities there is a marked difference. The % of positive reactors is always appreciably higher in an urban community than in a rural one. This difference is too large and too consistent to be one of mere coincidence. Cases tested at the tuberculosis dispensaries have not been included as they are of necessity a special sample of the population.

The Moloney results are in a direct contrast to the Schick results -- where the 'S+' decreases with age and is higher in a rural rather than in an urban community.

Moloney Reaction in relation to the Schick Reaction.

The diagrams relating to age and locality distribution indicate how Moloney and Schick tests have opposite meanings. This relationship was more fully studied.

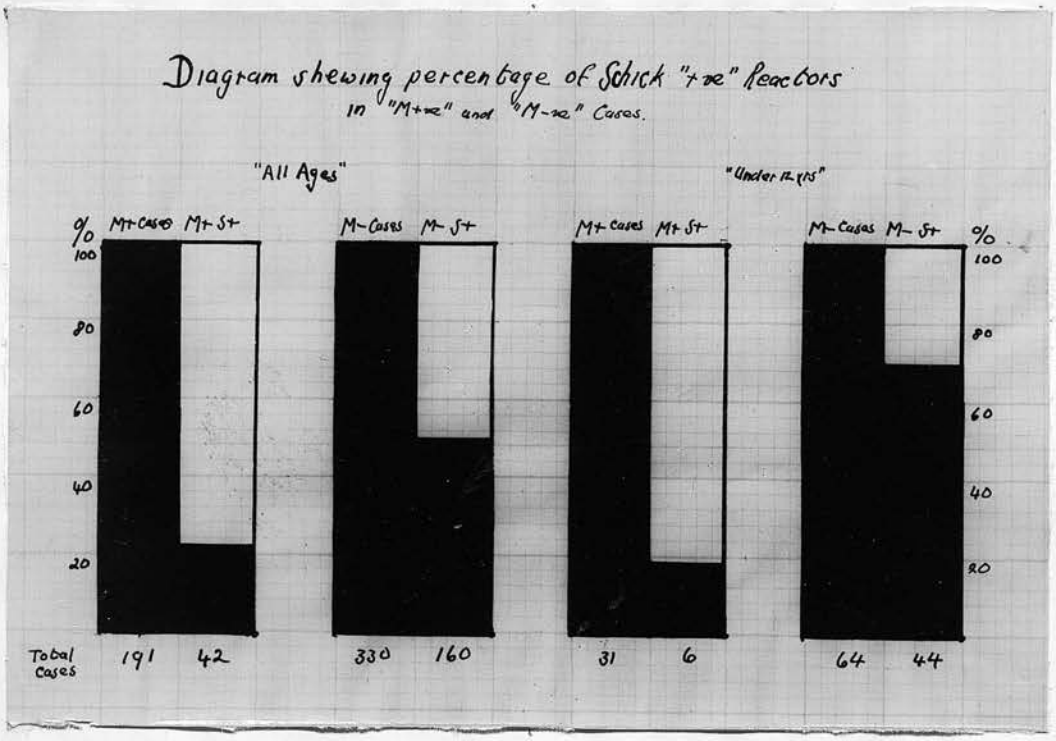


Diagram shewing percentage of Schick "+ve" Reactors
in "M+ve" and "M-ve" Cases.
"All Ages"
"Under 12 yrs"
% 100 80 60 40 20
M+ Cases
M+ S+
M- Cases
M- S+
Total Cases
191
42
330
160
31
6
64
44

A Coincident Schick and Moloney test was performed on a large number of cases. The Moloney state is taken and the cases are divided into positive and negative groups and the % of 'S+' in each group has been determined and the results expressed in diagram. As it was thought that age might alter this relationship, the results were worked out first for all ages and then for 'under 12s'.

The diagrams show that the 'S+' cases are more than twice as common amongst the 'M-' group than the 'M+' group, and in the 'under 12s' over 3 times more common.

A 'M+' case has a 4 to 1 chance of being immune to diphtheria. A 'M-' has equal chances, but if under 12 a 3 to 1 chance of being susceptible.

But there is also a qualitative difference. The Schick result varies greatly in intensity, and presumably this gives some index of susceptibility to the disease. In estimating the intensity of the Schick test, the diameters of the redness at site of injection were measured, and the product of these diameters gave a figure which indicated the extent of the reaction. This method could give no idea of intensity of redness, so it was dropped, and the reactions classified as:-

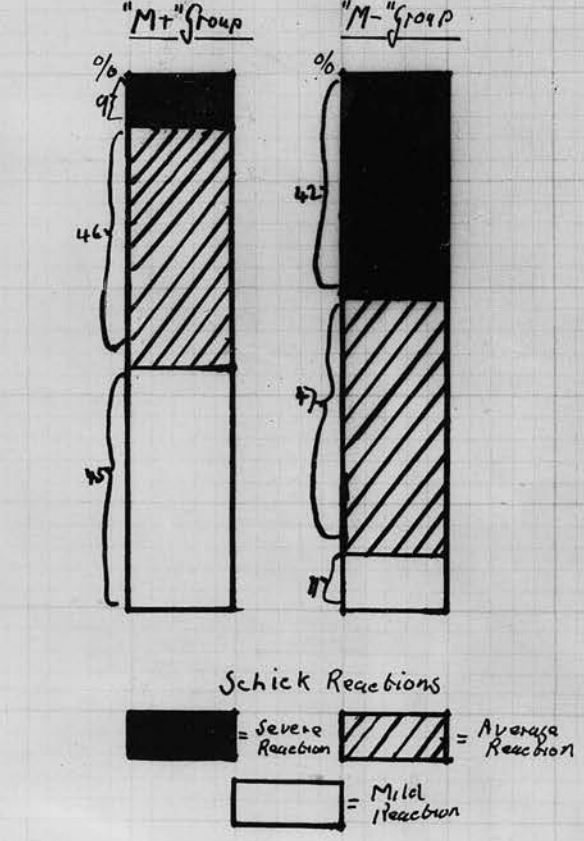
(1) slight

(2) severe

(3) average

purely on clinical grounds. Roughly speaking a

Diagram shewing percentage of "Severe", "Average", and "Faint" Schick Reactions in "M+" & "M-" Cases



reaction about 2 cm. by 1.5 cm. and salmon-pink in colour was considered to be an average reaction.

The % of each type of Schick in the 'M+' and 'M-' groups are expressed graphically and the diagrams show the following features.

They show that in each group the average Schick comprises about 50% of cases. The difference lies in the severe and mild reactions. In the 'M+' group most of the remainder are slight, while in the 'M-' group most are severe.

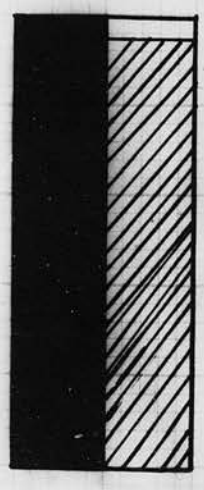
Thus in a 'M+' individual, the probability is that he is immune to diphtheria and if not completely immune, not likely to be very susceptible.

The permanence of the Moloney State.

This question is not of mere academic interest. In any system of dosage, especially where the number of injections is reduced to a minimum, there is sure to be a certain % of cases who are not satisfactorily immunised and who require further treatment. This lack of success is not likely to please and the method of immunisation will be very unpopular if in addition a severe reaction is subsequently caused.

Moloney tests were performed before immunisation and again at the same time as the final Schick test. The results are expressed diagramatically and show the total positives and negatives and the proportion of

Diagram indicating the effect of F.T. Treatment on the Moloney Reaction



= Total Moloney Tests performed

= unchanged reactions after F.T. Treatment [Both (+) & (−) cases]

[Diagram is based on approximately 200 cases tested before and after F.T. Treatment]

unchanged reactions.

There is no appreciable alteration in the Moloney state following injection. The differences can be explained by slight changes in technique.

The 2nd Moloney test was invariably performed more than 14 days after the injection of F.T. -- time enough for any sensitisation to develop. On the other hand the test was not repeated after one injection of F.T., so there might be some hint of desensitisation.

Other factors tend to make these observations merely approximate. While the writer of this thesis was present at all the testing, and the technique was the same in principle, the actual test was sometimes performed by different persons, and the individual factor cannot be ignored. Owing to questions of distance the readings of the test could not always be performed at the same time interval. Allowing for these sources of fallacy, a 94% correspondence-rate is a 'biological 100%'.

An opportunity arose to study this question under more strictly experimental conditions. Before recording details and results, mention has to be made of some kindred problems which were studied in the same subjects.

Several observers have noticed that serum sickness was more frequent and more severe in children immunised

with T.A.M. than in non-immunised children. Gordon and Creswell[30] give actual figures. They record serum reactions in 74.1% of cases which have previously received T.A.M., and only in 18% of cases who have not received this preparation. They suggest the use of F.T. or T.A.M. prepared from goat serum. These observations are not confined to Diphtheria, but also refer to Serum treatment of Scarlet Fever.

That this should occur is not surprising, when one remembers that T.A.M. mixture contains horse-serum; and injection therefore produces a state of hypersensitivity to the horse proteins.

In the preparation of Formol Toxoid horse serum is not used. Therefore toxoid ought not to produce this hypersensitivity in an individual -- another advantage over the serum containing immunising agents.

It was not possible to investigate this matter on a large scale, but small experiments were carried out to indicate an answer to the following questions:-

(1) The alteration in the Moloney state consequent on treatment with F.T.

(2) the question of serum-sensitiveness being produced by the different immunising agents.

The subjects chosen for these experiments were mainly infants under treatment for congenital syphilis or opthalmia neonatorum. Presumably sensitisation

in these infants and young children would not have developed, but in each case a preliminary test was performed before the child had been treated in any way.

Permanence of the Moloney Reaction.

The subjects were Moloney tested and then received one injection of F.T. (1 cc.). They were then retested at varying intervals. These tests were all performed by the writer personally. As the subjects were all in hospital, reading was particularly easy. The results are given on a table and show an almost perfect correspondence.

The conclusion is that the injection of F.T. has no effect on the Moloney reaction.

Age of Subject	Moloney before treatment	Moloney after Injection of F.T.	Interval between injection and re-testing.
5	-	-	16 days
9	+	+	16 days
13	+	+	16 days
2	-	-	16 days
3/52	-	-	15 days
7/12	-	-	17 and 42 days
3/52	-	-	20 and 45 days
10	-	-	40 and 65 days
10	+	(+	40 days
		(?+	60 days
4/12	-	-	39, 50 & 70 days
3/12	-	-	20 and 40 days
1	-	-	20 and 40 days
2	-	-	30, 50 & 70 days
4/52	-	-	30 days

Is a Sensitisation to Serum produced by the various immunising agents?

The importance of this question does not require any emphasis. Serum therapy is a feature of the modern treatment of many infectious diseases. Severe and dangerous reactions are rare, it is true, but much discomfort is caused by the milder ones. If diphtheria immunisation is to be performed on a large scale production of serum-sensitive individuals is a definite factor to be considered.

The children used for these observations were the same as previously -- congenital syphilitics and babies suffering from ophthalmia neonatorum. The material used for the experiments was diphtheria antitoxin and horse serum. This was diluted 1 in 5 with carbol-saline, and 0.2 cc. were injected intra-dermally. The readings were taken 1 hour, 3 hours and 24 hours after testing. Swelling, induration and redness were taken as a positive reaction.

All the subjects then received 1 cc. F.T. and the tests were repeated at varying intervals (never less than 10 days) and the same criterion of a positive reaction was taken.

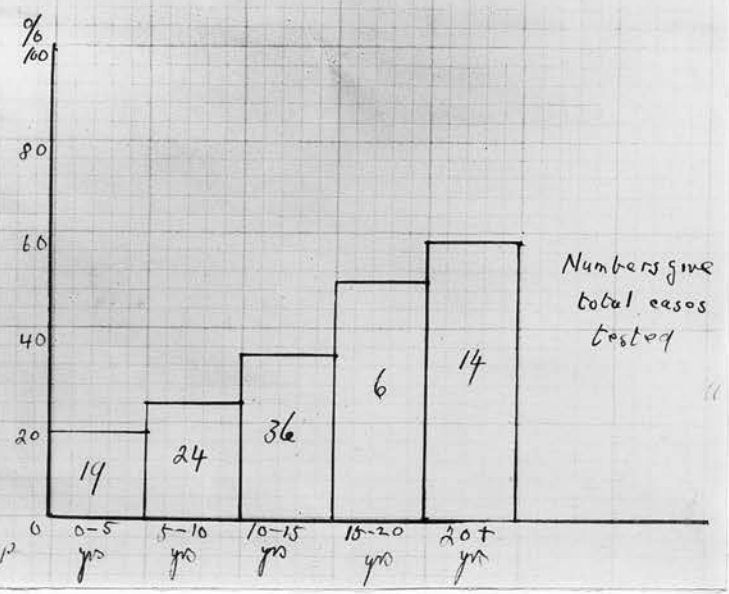
The cases then received 1 cc. T.A.M. and the tests were repeated, the interval again invariably being over 10 days. The following table indicates the results:-

Table showing results of serum sensitisation.

Age	Result of test before any injection.	Result of test after F.T.	Interval in days after injection.	Results of test after injection and T.A.M.	Interval in days after injection.
2 yrs.	-	-	21	Induration	14
1 yr.	-	? very slight induration	13	Marked induration	13
$\frac{7}{12}$	-	-	17	Induration	13
$\frac{4}{12}$	Slight Induration	-	17	Induration	13
10	-	-	13	Induration	15
10	-	-	16	Slight Induration	15
$\frac{7}{12}$	-	-	17	Induration	15
$\frac{3}{52}$	-	-	17	Induration	15
$\frac{2}{52}$	-	-	15		
2	-	-	15		
13	-	-	30		
9	-	-	16		
5	-	-	10		

Diagram shewing percentage of Tuberculin '+' Reactors amongst Somerset School Children and others [1933-34]

[Mantoux Test 0·1cc to 000 OT intradermally]



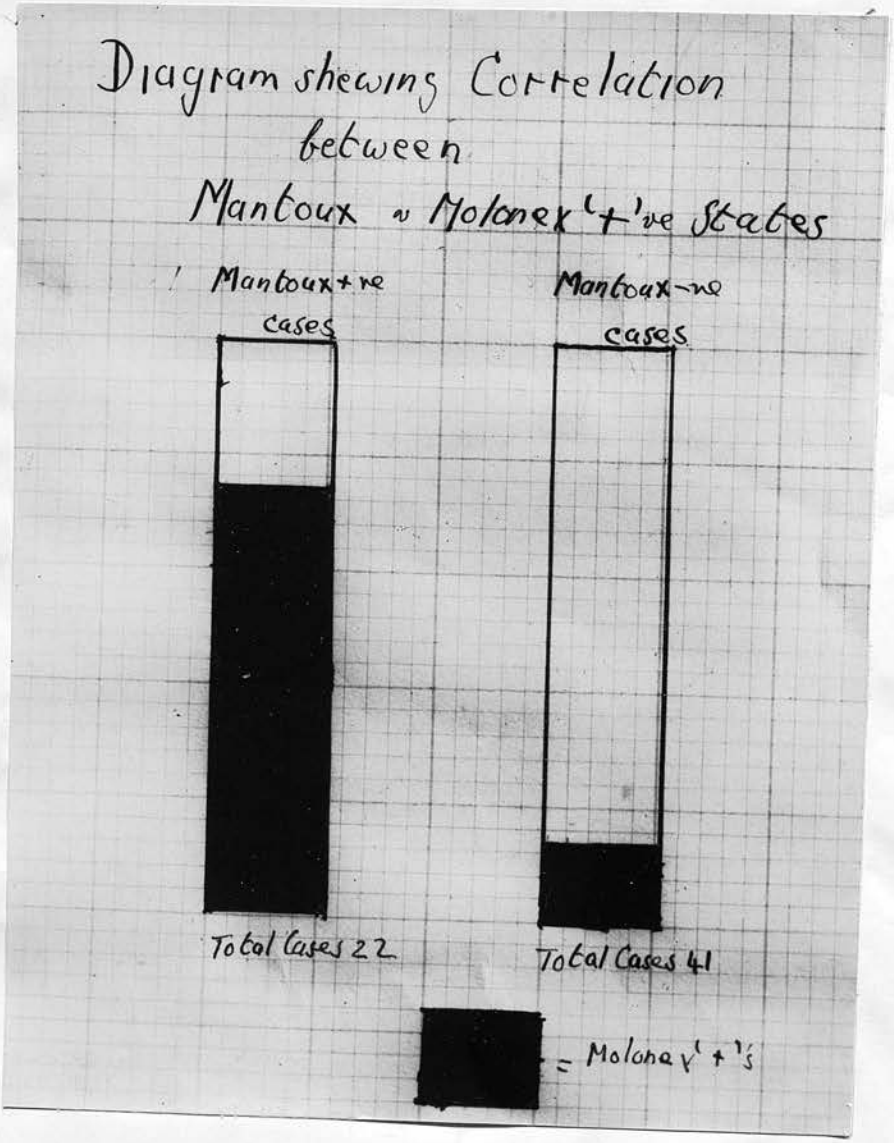
The number of cases is not large, but there is definite indication that the serum-containing agent produces sensitisation to sera used in treatment, while F.T. does not produce this effect.

When tabulating the Moloney results 2 features were noticed -- The % of positive reactors rises steadily with age -- and it is appreciably larger in cases coming from urban as compared with rural districts.

In considerating what the Moloney reaction is -- and therefore its use -- these 2 facts must be taken into consideration.

A similar distribution, both with regard to age and locality, is found in tuberculin sensitivity. A diagram, prepared from results obtained from testing school-children and others in Somerset is shown to emphasize this point. [The subjects of the test were T.B. contacts (mainly to T.B. -ve cases) and children seen at school inspection who, however remotely, might be suspected of T.B. -- a sample of population where one finds if anything a low % of tuberculin +ve reactors and would expect a low % of 'M+' reactors. The test was Mantoux's, one dose of 0.1 cc. of Old Tuberculin intradermally. Without being completely accurate this gives an index of tuberculisation.

Diagram shewing Correlation between Mantoux v Molonex '+'ve States



The diagram is, of course, not identical, but it shows the same form as one obtained from the Moloney results.

Another way of emphasizing this similarity was also tried. Moloney tests were done on children at the same time as the routine tuberculin test. From the nature of things, these tests were mainly on the population which is likely to be negative to both tests, i.e. rural school-children.

The Mantoux and Moloney results were read within 48 hours in most cases. The usual type of diagram has been prepared. Cases are divided into 2 groups 'Mantoux +' and 'Mantoux -' and the % of 'M+'s in each group shown. It is much larger in the Mantoux '+ve' group.

The numbers are too small to be dogmatic, but the two facts -- the similarity of the diagram and the fact that the Mantoux and Moloney tests seem to run parallel in the same young children -- (selected from a population where a '+' result to either test is comparatively rare), does suggest a common factor.

A positive tuberculin test indicates tuberculisation. It would seem that a '+' Moloney indicates 'diphtheriasation', an index of immunity. If this be the case the question what is the change produced by the formalin on the diphtheria toxin.

The Moloney test has been used in this investigation, and it must be admitted, very frequently mentioned in the account. Certain facts have been deduced from the various observations and experiments, and their importance has now to be discussed. It is not that the Moloney test in itself is important, but it is an index of sensitivity to perhaps the most efficient immunising agent. It is not possible to state dogmatically what is the cause of this sensitiveness, but the information obtained permits some interesting conclusions.

In a thesis concerned with matters practical in routine immunisation, it is not necessary to give details of manufacture. The method consists of allowing a weak formalin solution (0.3%) to act on Diphtheria Toxin (400 M.L.D. per cc.) at 37°C, causing the disappearance of its toxic, but not antigenic, properties.

Various chemical theories have been advanced to explain the change wrought by the formalin on the Diphtheria toxin. Its change in action is dramatic, e.g. $\frac{1}{50}$ M.L.D. of Toxin give a '+' Schick reaction; 4 M.L.D. after treatment with formalin may cause no reaction in a susceptible. Hewitt[31] suggested the following explanation of the change:-

(1) There is a disappearance of a fraction of the amino nitrogen of the toxin molecule or reaction with other groups of the molecule.

(2) There may be a union of two or more toxin molecules.

(3) There may be reduction, oxidation or combination with broth materials.

The change in the toxin is only one side of the problem. The other is the susceptibility which is present in the individual. The first thought is that the condition is in the nature of serum sensitisation. The changes occurring in the Moloney reaction are not unlike those noticed in tests for serum sensitisation, 0.2 cc. was enough in one case to cause oedema of the whole arm. Also the illness caused by injection of the toxoid has similarities to serum sickness itself.

It does not appear to depend on the quantity of toxoid injected. The experience of the use of the 'detector doses' confirms this point as in only 1 case did a reaction occur after 1 cc., where there was more after 0.1 cc. It is unlikely that, if quantity was an important factor, the threshold dose for a reaction should not in many cases be between 0.1 cc. and 1 cc.

Against this the Moloney takes about 24 hours to develop and after injection of F.T. the illness never shows such classical features as joint swellings, urticarial rashes. It is more nearly akin to the condition found after enteric inoculation.

There is more conclusive evidence that this is not a serum reaction. Formol toxoid is generally given

in two or more doses, the interval being over 10 days. It has never been reported that there were more reactions after a second dose than after a first. This was the experience in the present investigation and was further confirmed by Moloney testing before and after treatment. Allowing for unavoidable differences in technique, there was no change in the Moloney condition of the patient. The reverse also holds. It was not possible to desensitise the subject.

Another point is shown by the testing with horse serum. No sensitiveness to ordinary sera in therapy was produced. The liability to suffer from a reaction is therefore unlikely to be one of serum sensitisation.

There are now several positive facts. The age distribution gives one definite point. The incidence increases with age, indicating that the 'property', whatever it is, is an acquired one. The fact that it is more common in urban as compared with rural districts shows that acquisition depends on contact with human beings.

In age and locality distribution it is similar to tuberculin sensitivity. Also in individual cases, there is a much greater chance of a Tuberculin '+' individual being sensitive to F.T. than a Tuberculin -ve individual. These facts suggest some common factor in the production of the two conditions.

A positive tuberculin test is now recognised to

be evidence of previous infection with the tubercle bacillus causing sensitisation of the tissues. In practice it often means that resistence against the organism has developed. Arguing from analogy this property of reaction to F.T. ought to mean previous infection with the B. Diphtheria, with the development of resistence. One is luckier in the case of diphtheria than in the case of tuberculosis. There is a satisfactory index of susceptibility -- the Schick test. In actual fact, the Moloney and Schick test run counter to each other, confirming the view that sensitivity means development of resistence.

The modern view on the epidemiology of diphtheria lays stress on the existence of latent infections and immunisation. Diphtheria has always been considered to be a disease of local lesions and a general toxaemia -- both of which cleared up completely. Could it be that it is more in the nature of tuberculosis, where there is the persistence of some subclinical focus of infection, with some resulting change in the tissues?

This question is not completely answered in this thesis, but the evidence suggests the possibility of such an explanation.

Numerous gaps are present; it has not been determined whether the above results are specific to Formol Toxoid obtained from diphtheria toxin. But it was thought worth while to record the various facts observed and discuss their possible meaning.

ADDENDUM.

Sandhill Park.

Since the completion of the thesis it has been possible to study a small epidemic of diphtheria in an immunised institution. It must be remembered that the inmates are from the County of Somerset, essentially a rural district -- which normally would be a very susceptible community.

The first case occurred in a male aged 20 who was naturally 'S-' on October 4th, 1935. It was a mild case receiving 8000 units D.A.T. and the patient completely recovered by November 15th. The 2nd case occurred in a female aged 19 on October 14th. There was no connection with the first case. This girl was Schick -ve in June, following treatment with F.T. Swabs were negative, but she was clinically a definite case and received 10,000 units D.A.T. The other cases showed no clinical signs of symptoms, but the swab was '+'.

(1) Girl, aged 20, had slight tonsilitis & Pharyngitis with a positive swab on October 18th.

(2) Girl, aged 15, complained of sore throat, but no rise in temperature. The swab was '+' on Nov. 13th. She had clinical diphtheria in January, 1935.

(3) A girl, aged 14, also had a sore throat. The swab was '+'. She also had had clinical diphtheria in January 1935.

In addition there was a case of laryngeal diphtheria.

The institution, as has been already stated, consisted of a mansion and well-ventilated villas. It might be argued that the good hygiene was alone sufficient to prevent the spread of infection. But at the same time as the single cases of diphtheria were occurring there was a 'wave' of mild tonsilitis and pharyngitis -- too mild for the victims to be reported as sick, but showing that droplet infection was taking place.

Clinically two definite cases occurred, so the organisms were presumably virulent. There was therefore the introduction of pathogenic organisms into a community which would normally be highly susceptible -- and where, in spite of excellent hygienic conditions, droplet infection was occurring.

The fact that only 2 clinical cases occurred emphasizes the value of the immunisation, rather than detracts from it. It shows a high degree of success in face of a real danger -- which is perhaps more convincing than complete success against an assumed but unproved danger.

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